CLONING AND STABILITY STUDIES OF INDIVIDUAL AND TANDEM DYSTROPHIN ROD DOMAINS

Lena Pittman[1*], Laleh Saadat[2] and Nick Menhart[2]

[1]Wilbur Wright College, [2]Division of Biology, Illinois Institute of Technology

Dystrophin is the protein that is defective in Duchenne Muscular Dystrophy, a common fatal genetic disease characterized by muscle deterioration. This long, rod--shaped protein is thought to link the myocyte membrane to the machinery of muscle function, the myofibrils, and by doing so provides essential stabilization necessary for long-term survival of this tissue. Because myocytes experience large changes in shape and aspect ratio during repeated contraction/relaxation cycles, these two attachment points experience large relative movements, and so a completely rigid rod would not seem suitable.

The dystrophin rod is consists of 24 so-called spectrin type repeat, STR, motifs, a smaller ~ 5nm long rod domain. These are linked in tandem to produce the ~200 nm long dystrophin molecule. In addition to these rod regions, sequence homology analysis identified 4 motifs in the molecule that break up the STR regions and which do not seem to be identified as any known protein domain type. These regions are glycine and proline rich, and have been hypothesized to form unstructured hinges that allow articulation of the more rigid rod portions of the molecule. It has been shown that at least one such putative hinge region is essential to proper minimal function in mouse gene therapy models. However, there is no biophysical evidence of any excess flexibility associated with these non-homologous regions, so whether or not they are in fact hinges is not known.

We have undertaken a project to produce tandem and single STR motifs spanning the dystrophin molecule, including STRs linked by both putative hinge and non-hinge regions. Evidence of excess flexibility is being sought by thermodynamic stability analysis via CD and fluorescence, as well as by other biophysical techniques such as ultracentrifugation and SAXS. Data collected indicates that flexible regions may extend beyond the boundaries of the putative hinge regions, and into the flanking STR motifs.

[*] Lena Pitmann is supported by ILSAMP program through Wilbur Wright College.